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14. ABSTRACT

The purpose of this study is to examine neurovascular coupling in multiple sclerosis (MS) using a novel, noninvasive imaging technique for the simultaneous acquisition of both electrical (EEG) and blood volume and blood oxygen-based (NIRS and fMRI) signals, and to use the results to help optimize blood oxygen level dependent (BOLD) fMRI analyses of brain activity. Participants will be patients with MS (n=25) and healthy demographically matched controls (n=25) who will undergo standardized evaluations and imaging using combined EEG-NIRS-fMRI. EEG-NIRS data will be used to construct maps of neurovascular coupling parameters that will be applied to fMRI activation maps. Differences in neurovascular coupling and fMRI activation will be examined within and between groups. To date, we have refined the experimental set-up for the combined simultaneous acquisition of EEG-NIRS-fMRI and overcome a number of technical challenges, and acquired simultaneous EEG-NIRS-fMRI data in one participant. We have created cognitive and motor tasks that are amenable to analysis in all three imaging modalities and that activate expected brain circuitry. Recruitment is ongoing, with 19 patients and 27 controls active in our multi-stage recruitment process.

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1. Introduction

Background: Current wisdom suggests that the brain's adaptive capacity for functional reorganization helps mitigate effects of the progressive structural changes in the brain and spinal cord in MS. However, a critical reading of the fMRI literature in MS identifies some conflicting results. In fact, once thought to be indicative of one of the critical "missing links" that helps explain the clinical-radiological paradox in MS – the disconnect between extent of CNS damage and degree of impairment or disability – the fMRI literature now appears less clear and convincing. One reason for this is likely the fundamental limitation that blood oxygen level dependent (BOLD) fMRI indirectly estimates neuronal activity based on changes in blood flow and blood oxygenation. As such, it rests on the assumption that neuronal activity and vascular activity are intimately and reliably linked, and that this "neurovascular coupling" can be assumed to be constant across healthy and disease states. The assumption of neurovascular coupling must be challenged in any disease state that can or does affect one or more components of the neurovascular unit due either to the disease itself or associated factors such as medications.
Objective/Hypothesis: To examine neurovascular coupling in MS using a novel, noninvasive imaging technique for the simultaneous acquisition of both electrical (electroencephalogram (EEG)) and blood volume and blood oxygen-based (near-infrared spectroscopy (NIRS), functional MRI (fMRI)) signals, and to use the results to help optimize BOLD fMRI analyses of brain activity. **Study Design:** Participants will be patients with MS (n=25) and healthy demographically matched controls (n=25) who will undergo standardized evaluations and imaging using combined EEG-NIRS and fMRI. EEG-NIRS data will be used to construct maps of neurovascular coupling parameters that will be applied to fMRI activation maps. Differences in neurovascular coupling and fMRI activation will be examined within and between groups.

2. Keywords

BOLD – blood oxygen level dependent
EEG – electroencephalography
NIRS – near-infrared spectroscopy
fMRI – functional MRI
MS – multiple sclerosis

3. Accomplishments

What are the major goals of the project?

The major goals of this project are to examine neurovascular coupling in MS using a novel, noninvasive imaging technique for the simultaneous acquisition of both electrical (EEG) and blood volume and blood oxygen-based (NIRS, fMRI) signals, and to use the results to help optimize BOLD fMRI analyses of brain activity.

What was accomplished under those goals?

In the first year, we (1) obtained IRB and HRPO approval, (2) implemented simultaneous acquisition of EEG-NIRS-fMRI, (3) trained team members, including developing and carrying out a seminar for study project members covering topics on the theory, measurement, and analysis methods for EEG and NIRS, and (4) initiated recruitment and enrollment. The outline of the seminar developed for the training and professional development of study team members (and other interested students and faculty) is shown in **Figure 1**. This is, to our knowledge, the first time EEG-NIRS-fMRI data have been acquired simultaneously and we have devoted considerable time and effort to the following:

- Designed and implemented cognitive and motor activation tasks amenable to analysis in all three imaging modalities.
- Tested 8- and 32-channel head coils because these coils have different fits around the head, a significant factor when planning cabling for EEG and NIRS.
- Optimized our procedures for the 32-channel head coil (advantageous for fMRI signal-to-noise), and optimized our structural and functional MRI acquisition parameters.
- Tested the activation tasks to ensure expected cognitive and motor circuitry activity on fMRI for purposes of validation. Both tasks produced expected activation at the single subject level (**Figure 2**).

- Refined the design of the adjustable MRI compatible head probe, developed by our team for simultaneous collection of NIRS and EEG signals (http://www.ncbi.nlm.nih.gov/pubmed/23377012) (**Figure 3**), including optimizing EEG electrode and NIRS optode montage based on brain regions activated by the tasks.
- Designed new electrodes for improved set-up and montage customization.
- Acquired head and brain atlas data for analysis in MRI, boundary element mesh, and tetrahedral volumetric mesh data formats.
- Refined forward and inverse models for EEG and NIRS.
- Relocated the EEG-NIRS set-up to the Advanced Imaging Center for integration with fMRI
- Acquired new license dongle for testing with multiple EEG amplifiers
- Developed electrode connector box to enable head-probe to be used on multiple EEG amplifiers
- Developed trigger signal amplifier for fMRI for temporal synchronization of recordings
- Purchased new NIRS optical fibers for increased number of detectors and for sources with co-localized wavelengths (was 2, now 4)
- Continued refinement of the signal processing pipeline
    - improved independent EEG and NIRS signal processing algorithms
    - improved combined EEG-NIRS synchronization algorithms
    - developed EEG-NIRS data analysis algorithms for N-back task
    - developed algorithms to obtain optical properties of tissue at any montage chosen
    - developed algorithm to substantiate choice of electrode and optode montage
    - developed algorithms to generate tomographic images of EEG and NIRS activity
- Developed training protocol and workflow checklist for all staff for experimental set-up and recording. Ran several dry-runs to get our time from initial set-up to final tear-down to under two hours with a minimum of three team members.

Because of the complexities of physically and functionally integrating the three imaging systems (EEG, NIRS and fMRI), we had to devote considerable time to the above tasks and obtain approval for some SOW and IRB changes. Our requested changes have been approved and, with final (HRPO) approval, received in September 2015, we are working to recruit our target N of 25 patients and 25 healthy controls, and have enrolled and scanned our first participant. Our recruitment process involves several stages, starting with an initial study contact, followed by a basic screen, a full screen, and finally scheduling and enrollment. Our current recruitment status is:

For MS patients:
32 patients have completed basic screen OR have been referred to study by area provider or self
22 passed basic screen and we have attempted/are attempting to contact for full screen
8 have completed full screen
19 are in active recruitment (screening/scheduling/enrollment process)

For healthy controls:
29 healthy control volunteers have been considered for enrollment
8 have completed full screen
1 healthy control enrolled and completed procedures
27 are in active recruitment (screening/scheduling/enrollment process)

What opportunities for training and professional development did the project provide?

A seminar was developed for the training and professional development of study team members (and other interested students and faculty). The outline is shown in **Figure 1**.

How were the results disseminated to communities of interest?

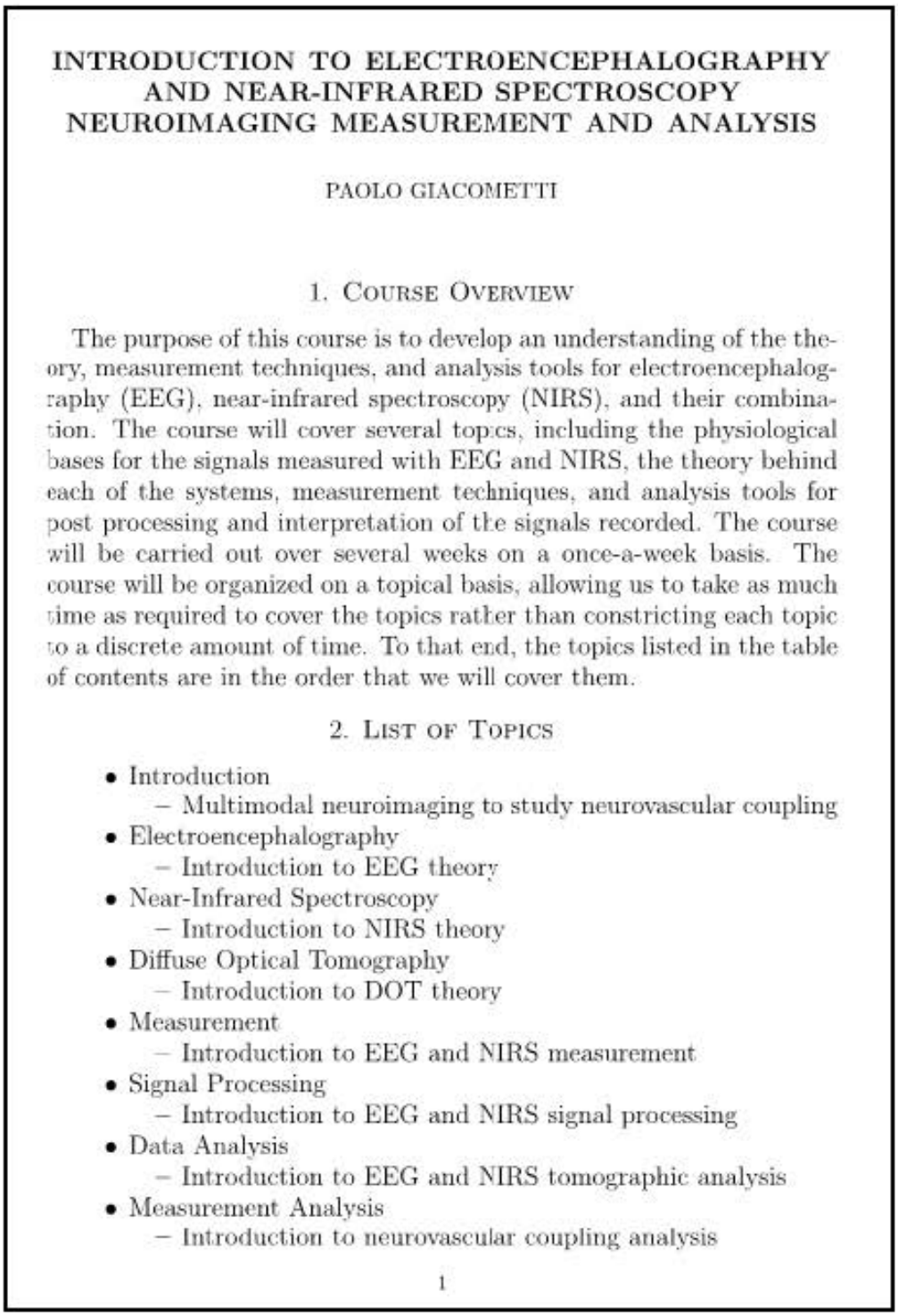
Nothing to report

Plans for next reporting period to accomplish the goals and objectives.

During the next reporting period, we will continue screening, recruiting, enrolling and scanning patients and controls, and will continue to refine the data processing pipeline as scans are completed. We had intended to spend an initial

six months on EEG-NIRS-fMRI set-up and then recruit/scan 5 controls and 5 patients in each of quarters 3 and 4, but instead it took 10 months to refine and implement the set-up and obtain all SOW, IRB and HRPO approvals for the necessary revisions. We will therefore devote significant effort to catching up on enrollment in the next quarters. Fortunately, we already have 46 potential participants in active recruitment, and our total target N is 50.

Figure 1. Course outline



INTRODUCTION TO ELECTROENCEPHALOGRAPHY AND NEAR-INFRARED SPECTROSCOPY NEUROIMAGING MEASUREMENT AND ANALYSIS

PAOLO GIACOMETTI

1. Course Overview

The purpose of this course is to develop an understanding of the theory, measurement techniques, and analysis tools for electroencephalography (EEG), near-infrared spectroscopy (NIRS), and their combination. The course will cover several topics, including the physiological bases for the signals measured with EEG and NIRS, the theory behind each of the systems, measurement techniques, and analysis tools for post processing and interpretation of the signals recorded. The course will be carried out over several weeks on a once-a-week basis. The course will be organized on a topical basis, allowing us to take as much time as required to cover the topics rather than constricting each topic to a discrete amount of time. To that end, the topics listed in the table of contents are in the order that we will cover them.

2. List of Topics

- Introduction
  - Multimodal neuroimaging to study neurovascular coupling
- Electroencephalography
  - Introduction to EEG theory
- Near-Infrared Spectroscopy
  - Introduction to NIRS theory
- Diffuse Optical Tomography
  - Introduction to DOT theory
- Measurement
  - Introduction to EEG and NIRS measurement
- Signal Processing
  - Introduction to EEG and NIRS signal processing
- Data Analysis
  - Introduction to EEG and NIRS tomographic analysis
- Measurement Analysis
  - Introduction to neurovascular coupling analysis

1

Figure 2. Statistical parametric maps for the cognitive and motor activation tasks in a single healthy control, showing relatively greater signal in bilateral frontal and parietal cortex for the working memory task relative to rest ((A) 2-Back > 0-Back contrast, $p_{FWE} < 0.05$) and contralateral sensorimotor cortex for the finger tapping task ((B) Right Hand > Rest contrast, $p_{FWE} < 0.05$). Data are presented in neurologic orientation (left = left).

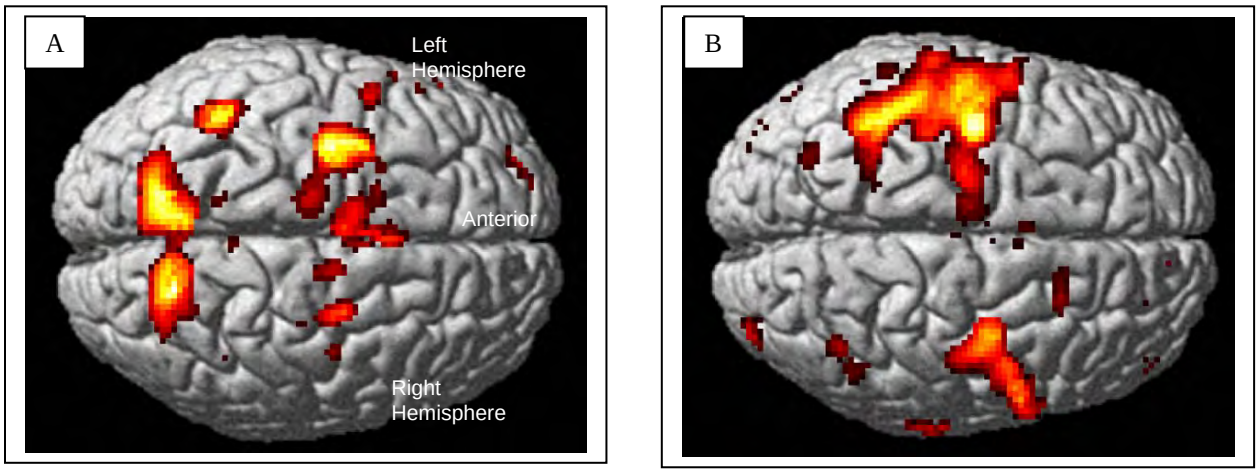
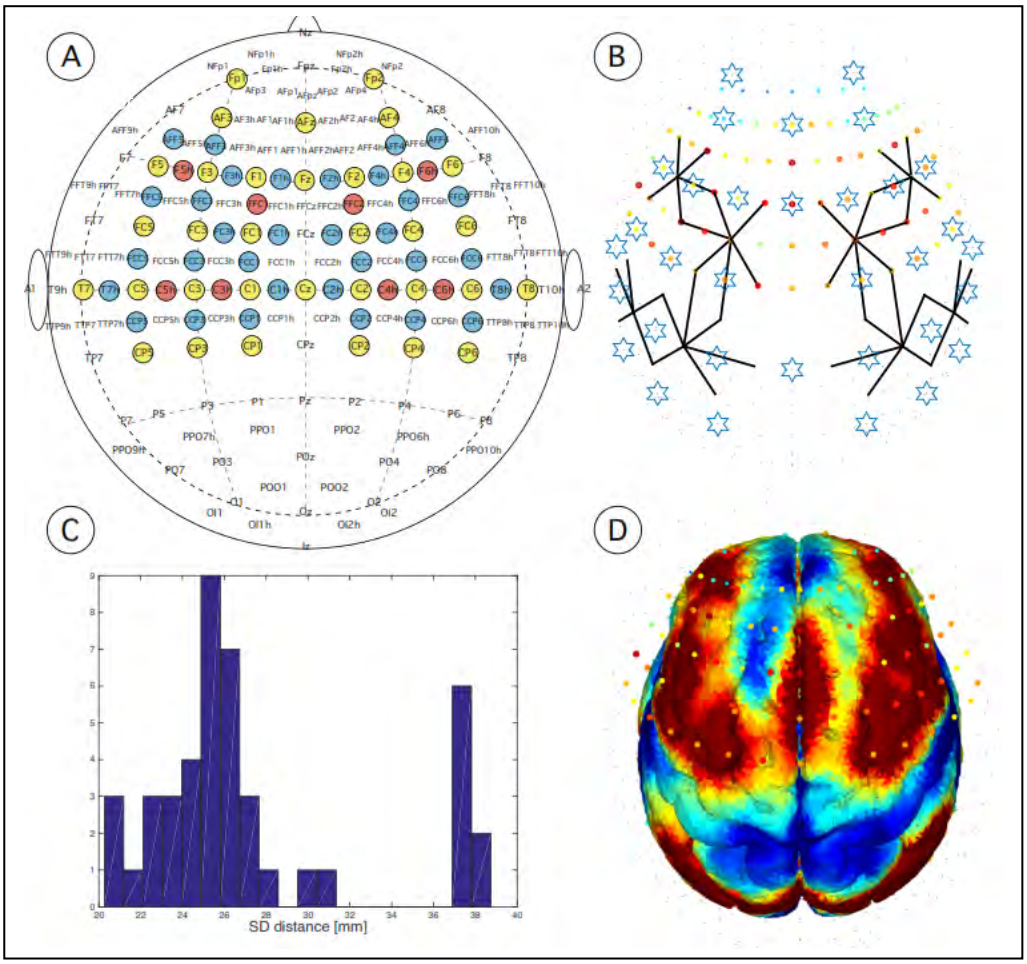


Figure 3. Head probe montage, showing the optode and electrode placements over frontal and parietal regions activated by the cognitive and motor tasks (A), the regional optode couplings (B) and distance variations (C), and the frontal and parietal regions activated by the working memory task (D).



4. Impact

What was the impact on the development of the principal disciplines of the project?

During this first year of the project, we have designed and implemented a means of acquiring simultaneous multimodal EEG-NIRS-fMRI data in humans. This will enable our group, and other neuroscientists in future, to test for abnormalities of neurovascular coupling that may contribute to neurological disease and dysfunction in humans, and to obtain neurovascular coupling data that can be used to optimize analysis of functional MRI data.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/ Problems

Changes in approach and reasons for change

We obtained DOD approval during the first year of the study to switch from our original plan of acquiring EEG-NIRS separately from the fMRI data to acquiring all three modalities simultaneously. While this increased the technical complexity of the project substantially, it will also enable us to improve significantly our assessment of the link between neurovascular coupling and fMRI signals in MS, and greatly facilitate use of the EEG-NIRS data to optimize the fMRI analyses. We also obtained DOD approval to recruit a broader range of MS subtypes to facilitate recruitment and increase ecological validity of the results.

Actual or anticipate problems or delays and actions or plans to resolve them

We encountered a number of technical challenges related to acquiring simultaneous EEG-NIRS-fMRI, e.g., compatibility of the electrodes with the MR environment, triggering of all three modalities to start data acquisition simultaneously. These were resolved by purchasing additional supplies and redesigning the set-up as needed. We also developed a detailed workflow checklist to enable the rapid set-up and tear-down of the equipment for each participant (as the equipment cannot be left in place when not in use).

Changes that had a significant impact on expenditures

We bought new NIRS optodes and increased our scan slots to two hours. The increased costs were offset by staffing changes (a postdoctoral fellow graduated and was replaced by a graduate student).

Significant changes in the use or care of human subjects, vertebrate animals, biohazards, and/or select agents

We have had no significant deviations, unexpected outcomes, or changes in the approved protocols.

6. Products

Publications, conference papers, and presentations

Nothing to report

Websites or other internet sites that disseminate results of the research activities

Nothing to report

Technologies or techniques

The NIRS-EEG head cap and integration of NIRS-EEG-fMRI are being refined as a result of this work, and further updates will be available as we proceed and publish on the technological aspects of the work.

Inventions, patent applications, and/or licenses

Dr. Diamond is the inventor on one patent and one patent application for technology that is being used in this study:
1. Diamond, S.G. System, Optode and Cap for Near-Infrared Diffuse-Optical Functional Neuroimaging. PCT/US09/41560 filed April 23, 2009, issued Sept. 23, 2013.
2. Diamond, S.G. and Giacometti, P. System and method for optode and electrode positioning cap for electroencephalography, diffuse optical imaging, and functional neuroimaging. PCT/US11/56566 filed Oct. 17, 2011.
This intellectual property (IP) is owned by Dartmouth.

Other products

Nothing to report

7. Participants and Other Collaborating Organizations

What individuals have worked on the project?

Name:	Heather Wishart, PhD
Project Role:	PI
Researcher Identifier (e.g, ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	As PI, Dr. Wishart oversees the conduct of the project and ensures data are collected and processed as planned.
Funding Support:	N/A

Name:	Sol Diamond, PhD
Project Role:	CoPI
Researcher Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Diamond ensures that the EEG-NIRS data acquisition proceeds according to plan and is integrated with the fMRI acquisition.
Funding Support:	N/A

Name:	Paolo Giacometti, PhD
Project Role:	Co-Investigator
Researcher Identifier:	N/A
Nearest person month worked:	7
Contribution to Project:	Dr. Giacometti taught the EEG-NIRS seminar for the study team members, designed the EEG-NIRS head cap, and worked to integrate EEG-NIRS with fMRI data collection. He has now graduated and left Dartmouth.
Funding Support:	N/A

Name:	Jenny Qiu
Project Role:	Graduate Student

Researcher Identifier: N/A
Nearest person month worked: 5
Contribution to Project: Ms. Qiu has worked to integrate the EEG-NIRS-fMRI set-up and contributes to data collection.
Funding Support: N/A

Name: Jennifer Randolph, MSc
Project Role: Database Manager and Research Associate
Researcher Identifier: N/A
Nearest person month worked: 2
Contribution to Project: Ms. Randolph contributes to developing the integrated EEG-NIRS-fMRI set-up and data collection, and manages the study database and recruitment/enrollment.
Funding Support: N/A

Name: James Ford, PhD
Project Role: Image Analyst
Researcher Identifier: N/A
Nearest person month worked: 1
Contribution to Project: Dr. Ford manages the fMRI data processing and integration with EEG-NIRS data analyses.
Funding Support: N/A

Name: Emily Geiger, BA
Project Role: Research Coordinator
Researcher Identifier: N/A
Nearest person month worked: 1
Contribution to Project: Ms. Geiger contributes to developing the integrated EEG-NIRS-fMRI set-up and data collection, and to recruitment/enrollment.
Funding Support: N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Heather Wishart – DOD Award W81XWH-09-1-0460 ended June 30, 2015.
Sol Diamond – no changes
Erik Kobylarz - Nat'l Inst of Biomed Imaging & Bioeng (NIBIB) (Design Optimization of Combined Magnetoencephalography and Susceptometry) – effort ended 6/30/15
Pachner - Biogen – (PEGIFN-beta) – ended 10/31/15

What other organizations were involved as partners?

Nothing to Report

8. Special Reporting Requirements: None.